CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE AG Am Strande 7 • 18055 Rostock • Germany

XXX

Order no.: xxx
Order received: xxx
Sample type: blood, filter card
Sample collection date: xxx
Report type: Final Report
Report date: xxx

Patient no.: **xxx**, First Name: **xxx**, Last Name: **xxx**
DOB: **xxx**, Sex: **female**, Your ref.: **xxx**

Test(s) requested: Whole Exome Sequencing (CentoXome GOLD®)

CLINICAL INFORMATION*

Abnormal brainstem MRI signal intensity, abnormality of the cerebral subcortex, ataxia, gait disturbance, lethargy, strabismus
*: Clinical information indicated above follows HPO nomenclature.

POSITIVE RESULT
Likely pathogenic variant identified

INTERPRETATION

By whole exome sequencing a homozygous likely pathogenic variant was identified in the NDUFS4 gene.
A genetic diagnosis of Mitochondrial complex I deficiency is confirmed.

RECOMMENDATIONS

- Parental carrier testing is recommended to confirm homozygosity in place of compound heterozygosity for a large deletion.
- Genetic counselling is recommended.

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RESULT SUMMARY

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
NDUFS4	Chr5(GRCh37):g.52978997_52979001dup NM_002495.2:c.474_478dup p.(Tyr160Cysfs*31) Exon 5	Hom	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A	gnomAD: 0.0000080 ESP: - 1000 G: - CentoMD: -	Frameshift Likely pathogenic (class 2)

Variant description based on Alamut Batch (latest database available). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations

VARIANT INTERPRETATION

NDUFS4, c.474_478dup p.(Tyr160Cysfs*31)

The NDUFS4 variant c.474_478dup p.(Tyr160Cysfs*31) creates a shift in the reading frame starting at codon 160. The new reading frame ends in a stop codon 30 positions downstream. This variant has been confirmed by Sanger sequencing. It is classified as likely pathogenic (class 2) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in the NDUFS4 gene are causative for Mitochondrial complex I deficiency with autosomal recessive inheritance. Isolated complex I deficiency is the most common enzymatic defect of the oxidative phosphorylation disorders. It causes a wide range of clinical disorders, ranging from lethal neonatal disease to adult-onset neurodegenerative disorders. Phenotypes include macrocephaly with progressive leukodystrophy, nonspecific encephalopathy, hypertrophic cardiomyopathy, myopathy, liver disease, Leigh syndrome, Leber hereditary optic neuropathy (535000), and some forms of Parkinson disease (OMIM®: 252010).

ANALYSIS STATISTICS WES

AVERAGE COVERAGE (X)	% TARGET BP COVERED					
	0X	≥ 1X	≥ 5X	≥ 10X	≥ 20X	≥ 50X
113.40	0.55	99.45	98.35	97.48	90.76	66.84

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

Class 1 – Pathogenic
Class 2 – Likely pathogenic
Class 3 – Variant of uncertain significance (VUS)
Class 4 – Likely benign
Class 5 – Benign
Additionally, other types of clinical relevant variants can be identified (e.g. risk factors, modifiers).

METHODS

RNA capture baits against approximately 60 Mb of the Human Exome (targeting >99% of regions in CCDS, RefSeq and Gencode databases) is used to enrich regions of interest from fragmented genomic DNA with Agilent's SureSelect Human All Exon V6 kit. The generated library is sequenced on an Illumina platform to obtain an average coverage depth of ~100x. Typically, ~97% of the targeted bases are covered >10x. An end to end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering out of low quality reads and probable artefacts, and subsequent annotation of variants, is applied. All disease causing variants reported in HGMD®, in ClinVar or in CentoMD® as well as all variants with minor allele frequency (MAF) of less than 1% in gnomAD database are considered. Evaluation is focused on coding exons along with flanking +/-20 intronic bases. All pertinent inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate eventually identified variants. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported.
Variants of relevance identified by NGS are continuously and individually in-house validated for quality aspects; those variants which meet our internal QC criteria (based on extensive validation processes) are not validated by Sanger.

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Targeted amplification of the entire mitochondrial genome is done using two overlapping long range PCRs. The amplicons are run on the Bioanalyzer to assess for any putative deletion within the mitochondrial genome. The amplified products are subsequently tagmented and Illumina compatible adapters ligated to generate libraries that are sequenced on Illumina platforms to an average sequencing depth of 1000x or more.
Raw sequence data analysis, including base calling, demultiplexing, alignment to the Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920) and variant calling are performed using validated in-house software. Following the base calling and primary filtering of low quality reads, standard Bioinformatics pipeline was implemented to annotate detected variants and to filter out probable artefacts. The pipeline confidently detects heteroplasmy levels down to 15%. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported.
Variants of relevance identified by NGS are continuously and individually in-house validated for quality aspects; those variants which meet our internal QC criteria (based on extensive validation processes) are not validated by Sanger.
Targeted sequencing was performed on both DNA strands of the relevant NDUFS4 gene region. The reference sequence is / sequences are: NDUFS4: NM_002495.3.

LIMITATIONS

Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical findings, additional testing should be considered.
Specific genetic events like copy number variants, translocations and repeat expansions may not be reliably detected with Exome Sequencing. In addition, due to limitations in technology, certain regions may either not be covered or may be poorly covered, where variants cannot be confidently detected.

ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE AG. The US Food and Drug Administration (FDA) has determined that clearance or approval of this method is not necessary and thus neither have been obtained. This test has been developed for clinical purposes. All test results are reviewed, interpreted and reported by our scientific and medical experts.

In line with ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Genetics in Medicine, 2016), we report incidental findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) only in the recommended genes for the recommended phenotypes.

To also exclude mistaken identity in your clinic, several guidelines recommend testing a second sample that is independently obtained from the proband. Please note that any further analysis will result in additional costs.

The classification of variants can change over the time. Please feel free to contact CENTOGENE (dmqc@centogene.com) in the future to determine if there have been any changes in classification of any reported variants.

DISCLAIMER

Any preparation and processing of a sample from patient material provided to CENTOGENE by a physician, clinical institute or a laboratory (by a "Partner") and the requested genetic and/or biochemical testing itself is based on the highest and most current scientific and analytical standards. However, in very few cases genetic or biochemical tests may not show the correct result, e.g. because of the quality of the material provided by a Partner to CENTOGENE or in cases where any test provided by CENTOGENE fails for unforeseeable or unknown reasons that cannot be influenced by CENTOGENE in advance. In such cases, CENTOGENE shall not be responsible and/or liable for the incomplete, potentially misleading or even wrong result of any testing if such issue could not be recognized by CENTOGENE in advance.

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